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## Synthesis and Characterization of Gadolinium Diethylenetriamine Pentaacetate-Folate

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### Abstract

In our preliminary radiopharmaceutical study, <sup>153</sup>Gd-DTPA-Folate has been successfully prepared, and tested for its bio-distribution. This continuing study is aimed at synthesizing and characterizing Gd-DTPA-Folate. The synthesis was started with the preparation of a derivative of folic acid, EDA-Folate, which was subsequently reacted with DTPA-Dianhydride to form DTPA-Folate. Finally, DTPA-Folate was reacted with Gd<sup>3+</sup> to result in Gd-DTPA-Folate, on a 0.1-10 gram scale, with a yield of 97.2%. Experimental data collected from characterization of purified synthetic products using ultraviolet, infrared, and mass spectroscopy, indicated the formation of Gd-O and Gd-N bonds leading to the formation of Gd-DTPA-Folate molecule.

**Keywords:** Folic acid; DTPA-Folate; Gd-DTPA-Folate; cancer; Magnetic Resonance Imaging.

### 1. Introduction

Nowadays cancer has been a major cause of death in the world, and the incidents of the disease tend to increase rapidly. About 12.7 million peoples have been diagnosed to have cancer every year, and 7.6 million of them were dead, mostly those having cervix and breast cancers. In Indonesia, cancer has been in the top ninth of the list of 50 death causing diseases<sup>1</sup>.

Magnetic Resonance Imaging (MRI) is able to detect cancer more rapidly than other diagnosis techniques can do. Furthermore, there are some more superiorities of MRI as a diagnosis method over the others, among others are its much smaller hazardous effect of radiation than those of such other methods as Computer Topography (CT) and

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Ultrasonography (US), and its non-invasive character<sup>2</sup>. MRI diagnosis technique has taken the advantage of using what so called contrast agents, which function to improve the quality of image produced. A contrast agent that has been recommended by the US FDA since 1988 is gadolinium-diethylene triamine pentaacetate (Gd-DTPA), with its brand name Magnevist<sup>3</sup>. This contrast agent cannot, however, be used to specifically detect cancers<sup>4</sup>. Thus, for this purpose a new type of contrast agents, targeted contrast agents, have been developed. This new type of contrast agents contains, in their molecular structures, a ligand having a high affinity to receptors on cancer cells. The ligand functions to direct the whole molecule of the contrast agent to go to the targets, the cancer cells, and thus do imaging specifically<sup>5</sup>. In the case of folic acid is the ligand, this has taken place through the folate-mediated endocytosis mechanism<sup>6</sup>.

So far, three radiopharmaceutical preparations have been synthesized by conjugating folic acid with radioactive-labelled ligands; all of them have been evaluated for their use as tumor markers. They are <sup>67</sup>Ga-deferoxamine-folate<sup>5</sup>, <sup>111</sup>In-DTPA-Folate<sup>5</sup>, and <sup>99</sup>Tc-DTPA-Folate<sup>7</sup>.

In our preliminary radiopharmaceutical study, <sup>153</sup>Gd-DTPA-Folate has been successfully prepared and tested for its bio-distribution<sup>8</sup>. This work is a continuing study aimed to synthesize and characterize Gd-DTPA-Folate, as a candidate for a targeted contrast agent.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Apparatus

This research employed instrumentation to include those for melting point determination (MP50 Melting point Mettler Toledo), a centrifuge, an ultraviolet spectrophotometer (UV HP 8452 A Shimadzu), an infrared spectrometer (FTIR Spectrum, Perkin Elmer), a liquid chromatograph-mass spectrometer (Waters LC-MS ESI-TOF), a high performance liquid chromatograph (Waters), a magnetic stirrer, a vacuum evaporator, a vacuum desiccator, and other general laboratory glass apparatus.

#### 2.1.1. Chemicals

Chemicals were purchased from Sigma Aldrich and Merck, and were of reagent- and or analytical grade. They included 2-propanol,  $\gamma$ -methyl glutamic acid, aquabidest, folic acid, hydrochloric acid, acetone, acetonitrile, diethyleter, diethylenetriaminepentaacetic dianhydride, dimethylsulfoxide, ethylenediamine, gadolinium chloride hexahydrate, hydrazine hydrate, potassium cyanide, methyl alcohol, sodium azide, sodium hydroxide, tertiary butyl nitrile, tetrahydrofurane, tetramethyl guanidine and trifluoroacetic anhydride.

### 2.2. Methods

#### 2.2.1. Synthesis of EDA-Folate

EDA-Folate was synthesized from folic acid through the method of five reaction steps. The five reactions steps were (consecutively): 1. Cyclization of the end part of the side chain of folic acid molecule; 2. Formation of a hydrazide from the reaction product of the first step reaction; 3. Formation of an azide from the hydrazide formed in the second step reaction; 4. Formation of a methyl ester of folic acid from the azide resulted in the third step reaction; and 5. Formation of EDA-Folate from the reaction of methyl ester formed in the fourth step reaction.

#### 2.2.1. Synthesis of DTPA-Folate ( $\gamma$ )

EDA-Folate ( $\gamma$ ) (0.1 g) was dissolved in dimethyl sulfoxide (5 mL). The resulted solution was then slowly added into a suspension of diethylenetriaminepentaacetic dianhydride (2 g) in dimethyl sulfoxide (1 mL). The result of the reaction mixture was cooled on an ice bath, neutralized with 2.4 N sodium hydroxide solution (1 mL), and then

centrifuged. The formed yellow precipitate was washed with acetonitrile and dissolved in water. The pH of the resulted solution was adjusted to 7.0 with a dilute hydrochloric acid solution. The produced light yellow solution was finally filtered, and evaporated to dryness, leaving a yellow solid product. The final reaction product was analyzed by TLC and HPLC. A concentrated aqueous solution of the product was passed onto a preparative reversed-phase chromatographic column using a stationary phase of octadecylsilane (ODS) and a mobile phase of methanol:water (1:9). The collected fractions were analyzed by HPLC, and those fractions containing DTPA-Folate were evaporated to get a pure DTPA-Folate product.

### 2.2.2. Synthesis of Gd-DTPA-Folate<sup>9</sup>

To 0.204 g ( $5.5 \times 10^{-2}$  mmole) of  $\text{GdCl}_3 \cdot 6 \text{H}_2\text{O}$  and 0.429 g ( $5 \times 10^{-2}$  mmole) of the synthesized DTPA-Folate in a round-bottom flask, 10 mL of aquabidest was added. The reaction mixture was then heated under reflux condensor for 2 hours. The resulted reaction mixture was neutralized, filtered, and dried in a freeze dryer. The produced yellow solid was analyzed by HPLC and TLC. A concentrated aqueous solution of the product was passed to preparative chromatographic column with a stationary phase of ODS and a mobile phase of methanol:water (1:9). Chromatographic fractions were collected and analyzed by HPLC and TLC. All the fractions containing Gd-DTPA-Folate were combined, evaporated, and the purified product was characterized using ultraviolet-, infrared-, and mass spectrometry. The melting point of the reaction product was also determined.

## 3. Results and Discussion

### 3.1. Synthesis of EDA-Folate

The MRI contrast agent Gd-DTPA-Folate being studied will be a new product developed from the earlier contrast agent Gd-DTPA, which has long been used in medical diagnosis. The new contrast agent is different from Gd-DTPA, in that the former will be able to detect cancer, while the latter cannot; Gd-DTPA is a general and non-specific contrast agent.

This has been made possible because the molecule of this particular new type of contrast agent has the folate group in it, which is able to direct the whole contrast agent molecule to go to the folate receptor (that is why this type of contrast agent is called targeted contrast agent) on the surface of cancer cells. In other words, the contrast agent detects the cancer cells. The interaction of folic acid with the folate receptor on the cancer cells occurs (expression on normal cells is less than that on cancer cells), through the folate-mediated endocytosis.

Gd-DTPA-Folate was formed from the reaction of  $\text{Gd}^{3+}$  with DTPA-Folate, which was freshly synthesized via the reaction between DTPA-dianhydride and EDA-Folate, which was previously prepared by adopting and modifying the long route but established method<sup>9</sup>. In this method, folic acid was converted consecutively into pyrofolate acid, pteroyl hydrazide, pteroyl azide, methyl ester of folic acid, and finally into EDA-Folate (Figure 1).

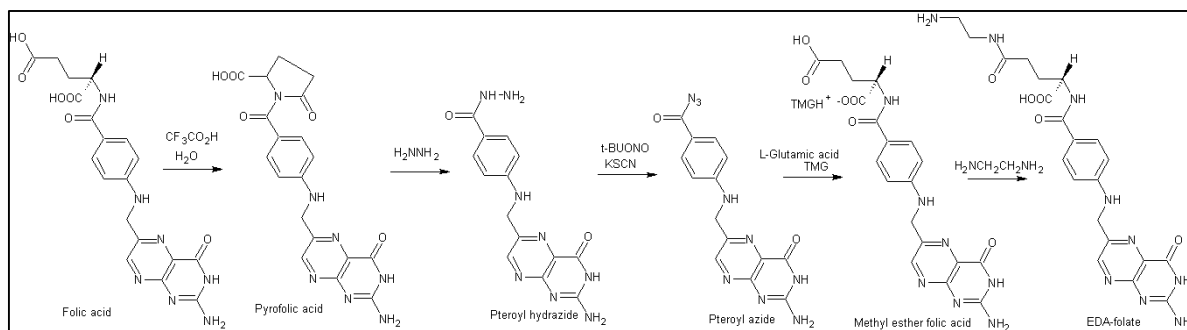


Fig. 1. Reaction steps in the synthesis of EDA-Folate from folic acid (Adopted from US 20070042970 Patent)<sup>9</sup>

Thus, the success in the preparation of EDA-Folate as an important precursor, depends on how each of the reaction

steps to form the intermediate was carried out. This includes optimization of the reaction conditions and purification process. Figure 2 below is a histogram showing the yield of each reaction step during the preparation of EDA-Folate from folic acid.

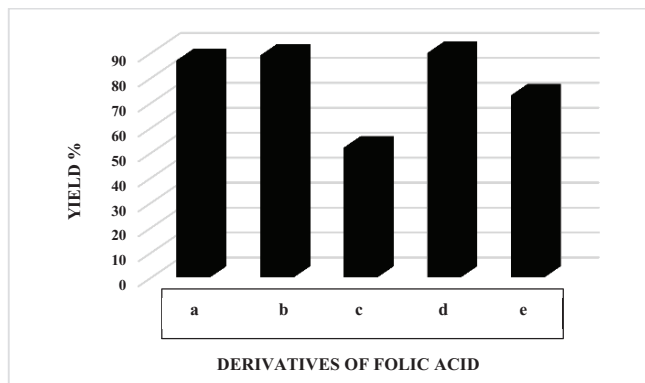


Fig. 2. Yield of reaction steps during the preparation of EDA-Folate from folic acid (derivates of folic acid). (a) Pyrofollic acid, (b) Pteroyl hydrazide, (c) Pteroyl azide, (d) Methyl ester folic acid ( $\gamma$ ), and (e) EDA-folate ( $\gamma$ ).

Figure 2 shows that the yield of four out of the five reaction steps were more than 70%, which are good. But, optimization of the conditions of the third reaction step (Figure 1), such as formation reaction of pteroyl hydrazide from pteroyl azide with a yield of 50%, still have to be done.

Apart from the yields of the reactions, purity of the reaction products is another important aspect. Thus, all the reaction products have been purified and analyzed for their purity by chromatographic methods.

### 3.2. Syntesis of DTPA-Folate

DTPA-Folate was synthesized through a substitution reaction of DTPA-dianhydride with EDA-Folate, which had been prepared freshly. The reaction involved an amidation of the primary amine in the EDA-Folate molecule with one of the carboxyl groups in the DTPA molecule. The molecular structure of the resulted DTPA-Folate is shown below in Figure 3.

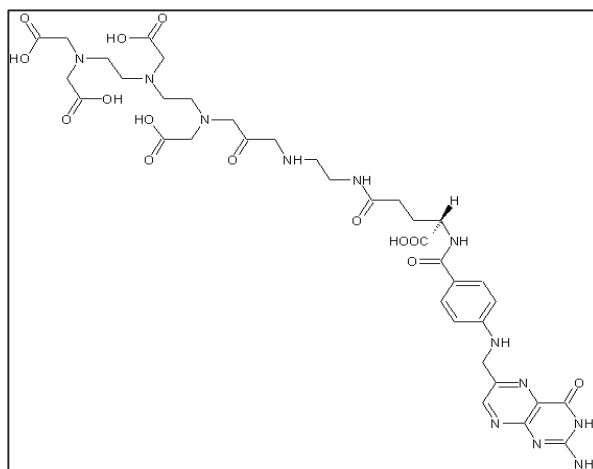


Fig. 3. Molecular structure of DTPA-Folate.

Purification of the produced DTPA-Folate (also EDA-Folate and DTPA-dianhydride which was done on a reversed-phase chromatographic column,  $C_{18}$ , with a mobile phase of a methanol:water, mixture (1:9) has resulted in a sufficiently pure product required for its characterization. Figure 4 below is a chromatogram of the purified product of DTPA-Folate.

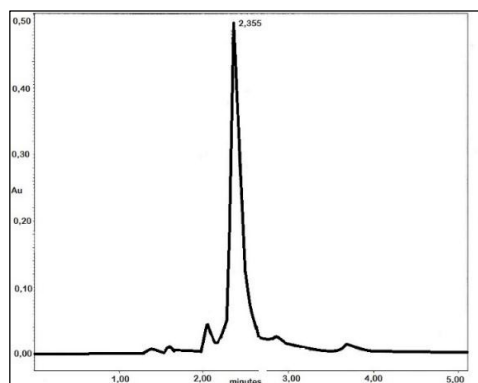


Fig. 4. HPLC chromatogram of the resulted DTPA-Folate. Chromatographic conditions were:  $C_{18}$  column, isocratic elution with a mobile phase of a mixture of acetonitrile: 0.3% TFA (2:8), flow rate of 1 mL/minute, ultraviolet detection at 280 nm.

The chromatogram shows a single peak at 2.4 minutes, indicating that the resulted DTPA-Folate was pure and thus can be used further for the synthesis of Gd-DTPA-Folate (Fig. 6)..

### 3.3. Synthesis Gd-DTPA-Folate

Gd-DTPA-Folate was synthesized via a complexation reaction of  $Gd^{3+}$  ion with DTPA-Folate that had been synthesized previously. Figure 5 below is a proposed molecular structure of the resulted complex.

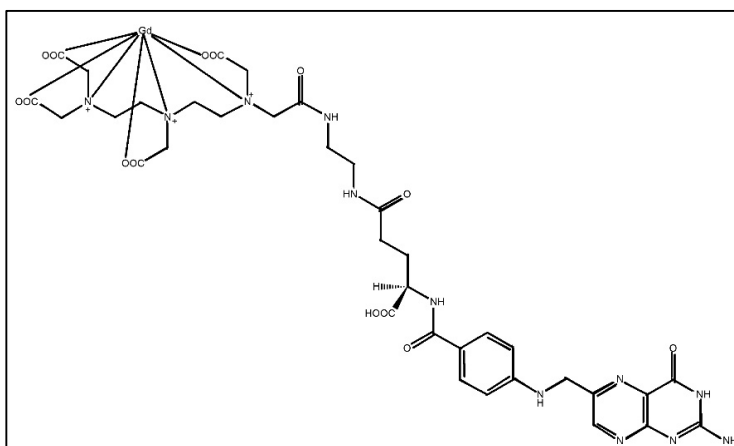


Fig. 5. A proposed molecular structure of the synthesized Gd-DTPA-Folate.

As shown in Figure 5, seven chemical bonds were formed from gadolinium atom, nitrogen atoms and oxygen atoms, consisting of four Gd-O and three Gd-N bonds. These had been made possible to occur because of the presence of the lone pair electrons on the nitrogen and oxygen atoms. The electron pairs were shared with gadolinium atom in the formation of the complex molecule, formation of Gd-O and Gd-N bonds.

In order the reaction to take place more rapidly and completely, one of the reactants,  $\text{Gd}^{3+}$  ion was added in excess, more than the required amount calculated based on reaction stoichiometry. Applying the selected experimental conditions, it was found that the reaction took place almost completely in two hours, to result in a yield of 97.2%.

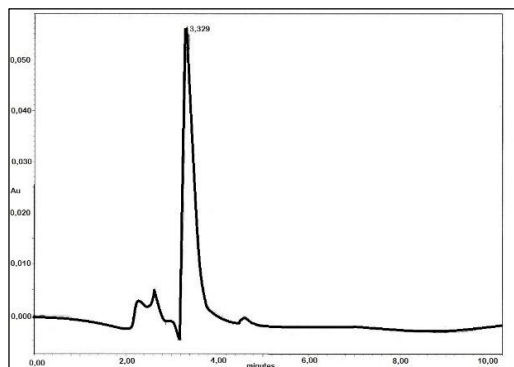


Fig. 6. HPLC chromatogram of the resulted Gd-DTPA-Folate. Chromatographic conditions were:  $\text{C}_{18}$  column, isocratic elution with a mobile phase of a mixture of acetonitrile: 0.3% TFA (2:8), flow rate of 1 mL/minute, ultraviolet detection at 280 nm.

Apart from the yields of the reactions, purity of the reaction products is another important aspect. Thus, all the reaction products have been purified and analyzed for their purity by chromatographic methods. Data on melting point of the synthesized products confirm that the products were pure as indicated by their melting points data (DTPA-Folate, 73.0 – 73.4°C; Gd-DTPA-Folate, 100.5 – 101.0°C).

Characterization of the synthesized contrast agent and the precursor was done by using different methods of spectroscopy; they were ultraviolet-, infrared-, and mass spectroscopy. The following is the ultraviolet spectra of the synthesized DTPA-Folate (Figure 7a).

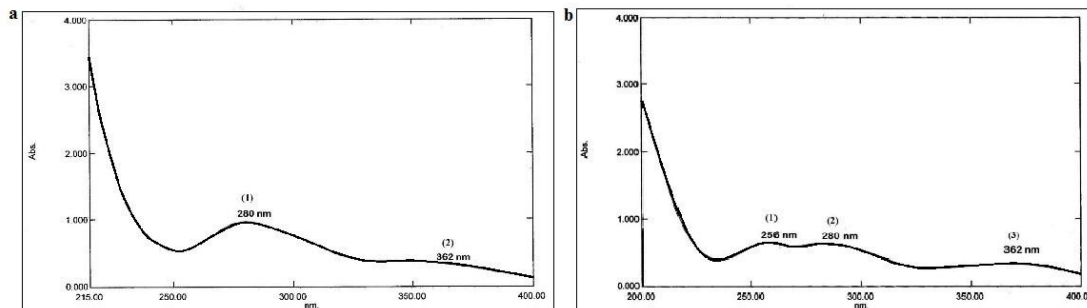


Fig. 7. Ultraviolet spectra of synthesized DTPA-Folate (a) and ultraviolet spectra of Gd-DTPA-Folate (b) in the range of 200–400 nm.

The ultraviolet spectra of the synthesized DTPA-Folate show an absorption peak at 280 nm originated from the  $\text{C}=\text{C}$  bonds of the benzene rings (electronic transition from  $\pi \rightarrow \pi^*$ ), and an absorption peak at 362 nm coming from the conjugated  $\text{C}=\text{O}$  bonds (electronic transition from  $n \rightarrow \pi^*$ ) (see the molecular structure of DTPA-Folate (Figure 3)).

Meanwhile, the ultraviolet spectra of the synthesized Gd-DTPA-Folate (Figure 7b) show an absorption peak at 256 nm, other than those on 280 nm and 362 nm. These latter two absorption peaks are present in the ultraviolet spectra of the synthesized DTPA-Folate<sup>10</sup>. From this comparison of the ultraviolet spectra of the two synthesized compounds it can be drawn a conclusion that the absorption peak at 256 nm originated from the formation of the complex compound or new bonds, the  $\text{Gd}-\text{O}$  and  $\text{Gd}-\text{N}$  bonds, because of hypsochromic effect (see the proposed molecular structural of Gd-DTPA-Folate in Figure 5). The higher intensity of the absorption peak of DTPA-Folate in the region of 272–278 nm was probably due to the presence of gadolinium atom in the molecule of Gd-DTPA-

Folate as well. Infrared spectra of the synthesized compounds are presented in Figure 8 below.

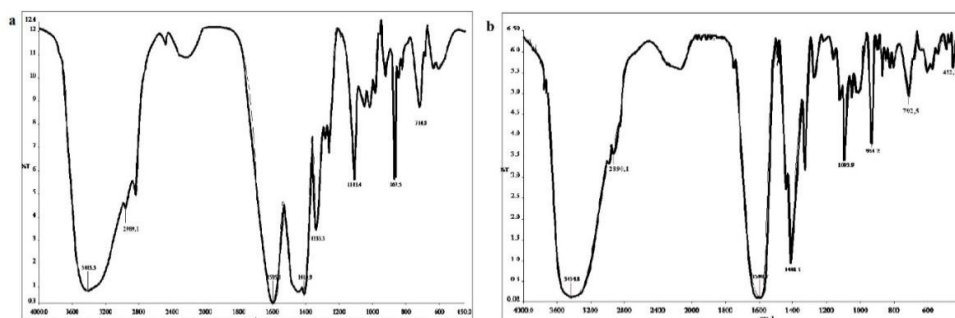


Fig. 8. Infrared spectra of the synthesized DTPA-Folate (a) and Gd-DTPA-Folate (b) the wave number range of 450–4,000  $\text{cm}^{-1}$ .

From a comparison between the infrared spectra of the synthesized DTPA-Folate (Fig. 8a) and that of the synthesized Gd-DTPA-Folate (Figure 8b) it can be seen the presence of absorptions at similar wave-numbers, both in the functional group region (4,000 – 1,400  $\text{cm}^{-1}$ ) and in the finger print region (1,400 – 400  $\text{cm}^{-1}$ ). The absorption peaks are: at 3,434.8  $\text{cm}^{-1}$ , originating from the stretching vibration of O-H and or N-H groups; at 2,890.1  $\text{cm}^{-1}$  from the stretching vibration of C-H groups; and at 934.2  $\text{cm}^{-1}$  from C-O and C-N groups; and at 792.5  $\text{cm}^{-1}$  from C-H groups. An identified difference is the presence of an absorption at 452.1  $\text{cm}^{-1}$  in the spectra of Gd-DTPA-Folate, but not in the spectra of DTPA-Folate, which probably comes from vibration of Gd-O bonds. Another difference between the two synthesized spectra is the lower absorption intensity of OH groups in Gd-DTPA-Folate at the wave number of 3,434.3  $\text{cm}^{-1}$  than that of in DTPA-Folate. This may be due to the binding of the OH groups in DTPA-Folate molecule by  $\text{Gd}^{3+}$ .

Mass spectra of the synthesized compounds are presented in Figure 9 below.

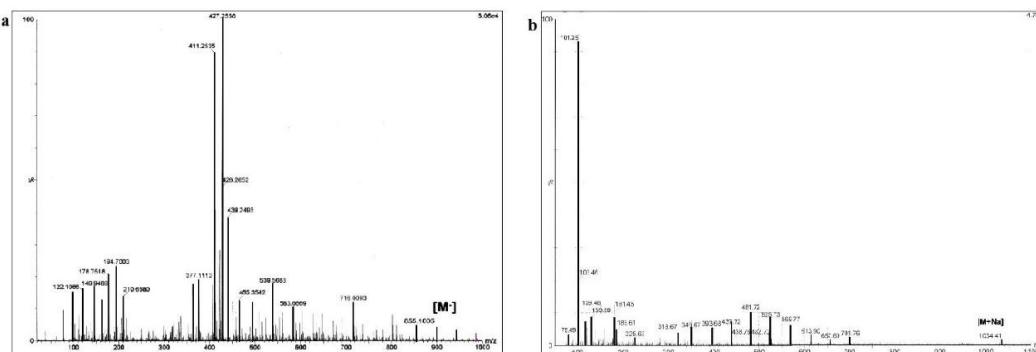


Fig. 9. Mass spectra of the synthesized DTPA-Folate (a) and Gd-DTPA-Folate (b), in the  $m/z$  range of 100–1,100.

Mass spectra the prepared DTPA-Folate (Fig. 9a) show the presence of a parent peak at an  $m/z$  of 855.1005, which corresponds to a calculated molecular mass of the compound. Meanwhile, mass spectra of the produced Gd-DTPA-Folate (Fig. 9b) show the presence of a parent peak at an  $m/z$  of 1034.41, which corresponds to the calculated molecular mass of the synthesized candidate for a targeted contrast agent, as its sodium salt.

## Conclusions

Based on the discussions given above, the conclusion of this study is that Gd-DTPA-Folate could successfully be synthesized in a good yield, from the reaction of gadolinium ion with DTPA-Folate, which was freshly prepared from EDA-Folate, which in turn was synthesized from folic acid, through several reaction steps. The Gd-DTPA-

Folate product was produced on a 0.1-10 gram scale, in the form of pale yellow crystals.

### Acknowledgements

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### References

1. WHO. 2013. *Cancer*. <http://www.who.int/topics/cancer/en> (accessed on July 18<sup>th</sup>, 2014).
2. Loreti, B and J. Bettmer. Determination of the MRI contrast agent Gd-DTPA by SEC-ICP-MS. *Springer-Verlag*, 2004:379:1050-1054.
3. Ding, Z., Y. Chen, Y. Sun, W. Zhang, Z. Huang and L. Zhang. Preparation of <sup>99m</sup>Tc-Gd-DTPA-Dimeglumine and Its Biologic Characteristics. *Journal of isotopes* 2000:22(3):149-155.
4. Gries, H., D. Rosenberg, H. J. Weinmann, U. Speck, W. Mutzel, G. A. Hayden and H. Pfeiffer. *Method to Enhance NMR Imaging using Chelated Paramagnetic Ions*. US Patent 4963344. 1990.
5. Wang, S., J. Luo, D. A. Lanrip, D. J. Waters, C. Mathias, M. A. Green, P. Fuchs and P. Low. Design and Synthesis of [<sup>111</sup>In]-DTPA-Folate for Use as a Tumor-targeted Radiopharmaceutical. *Bioconjugate Chemistry* 1997:8:673-679.
6. Zwicke, G., G. A. Mansoori and C. Jeffery. Utilizing the Folate Receptor for Active Targeting of Cancer Nanotherapeutics. *Nano Rev* 2012:3-10.
7. Mathias, C. J., D. Hubbers, P. S. Low and Green. 2000. Synthesis of <sup>99m</sup>Tc-DTPA-Folat and Its Evaluation as a Folate Receptor Targeted. *Bioconjugate Chem* 2000:11(2):253-257.
8. Gunawan, A. H, G. Yono and Maskur. Sintesis dan Karakterisasi Gd DTPA Folat sebagai MRI Contrast Agent dan Penyiapan Dokumen dan Karakterisasinya menggunakan Perunut Radioaktif <sup>153</sup>Gd-DTPA-Folat. *Jurnal Sains Materi Indonesia (Edisi Khusus Material untuk Kesehatan)* 2012:1-6.
9. Sunamoto, J., K. Ushio and H. Massaki. *Folate Modified Cholesterol Bearing Pullulan as a Drug Carrier* USP 20070042970A1. USA patent. 2007.
10. Wang, S., R. Lee, C. Mathias, M. A. Green and P. Low. Synthesis, Purification, and Tumor Cell Uptake of <sup>67</sup>Ga Deferoxamine Folate, a Potential Radiopharmaceutical for Tumor Imaging. *Bioconjugate Chemistry* 1996:7(1):56-62.